Final revised
~~August 19~~
March 1987

88-05384/

A SENSITIVE SCREENING BIOASSAY TECHNIQUE FOR THE TOXICOLOGICAL ASSESSMENT OF SMALL QUANTITIES OF CONTAMINATED BOTTOM OR SUSPENDED SEDIMENTS

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ABSTRACT

The biological impact assessment of sediment-bound contaminants is currently considered essential in addition to routine chemical characterization. Conventional methods of sediment bioassessment require relatively large quantities of the sample. When sample sizes are limited, however, these methods become ineffective. To overcome this problem, the Limited Sample Bioassay (LSB) technique was developed. Bioassays conducted with bottom and suspended particulates from the western, central and Arctic regions of Canada indicated that the newly developed LSB technique successfully evaluated the contaminant/nutrient impact on laboratory-grown cultures of algae. The LSB method produced results which were comparable to those from standard elutriate bioassays conducted with natural phytoplankton size assemblages as well as with laboratory-grown monocultures. The data suggest that the LSB can play an important role in providing a quick, simple, sensitive, and inexpensive screening technique for assessing the bioavailability of contaminants (or nutrients) from sediment of limited quantity.

INTRODUCTION

In the autumn of 1985, the biological impact assessment of Lake Diefenbaker sediments and suspended particulate matter collected from Alberta, Saskatchewan, and the Northwest Territories in Canada was carried out (Munawar et al., 1986). This study was challenging since the high cost of transportation, and the logistical problems of sample collection from remote Arctic sites, permitted the collection of only limited quantities of sediment sample. In other instances, limited quantities of samples resulted from the unavailability of sediment due to the poor patterns of deposition.

It should be noted that there are inherent difficulties in collecting large quantities of suspended particulate matter in any environment; so it is now a common practice to centrifuge hundreds or thousands of litres of water to obtain a few grams of the suspended matter. Since this is time-consuming and expensive, any procedure that would permit effective biological evaluation of small quantities of particulate matter (or sediment), would be of great value to researchers in the area of assessing the toxicity of sediment-bound contaminants.

It was necessary therefore, to devise a technique which would permit the biological assessment of very small samples of suspended particulate matter and sediment, even if it served only as an accurate screening technique. Hence this paper presents a new method for achieving such an objective. Therefore, the name

Limited Sample Bioassessment (LSB) has been adopted.

MATERIALS AND METHODS

Collection of Sediment and Suspended Particulate Matter

Ten samples were collected from five sites in Alberta, Saskatchewan and the Northwest Territories (Figure 1), of which six were suspended particulate matter (SP), and four were bottom sediments (BS). Bottom sediments were collected using a Ponar dredge, whereas an Alfa Laval continuous flow separator was used to isolate suspended particulates (Frank et al., 1979; Ongley and Blatchford, 1982). These samples were collected in 1984 and 1985, sub-sampled, labelled, and frozen until toxicity assessment was carried out. It is recognized that sediment should be submitted to toxicity assessment as soon as possible after collection (Brannon and Engler, 1977). However, the difficult circumstances resulting from sediment collection under arctic conditions over an extended period of time, and the significant period between sample collection and their utilization in biological assessment necessitated freezing as the best option for storage. Wet-weight sub-samples were taken from parent samples which ranged in size from 50 gm to 95 gm.

In 1986, three additional sediment samples were collected from Toronto Harbour (Ontario) using the methods described above. They were stored briefly at 4°C and two were used in comparative LSB studies, while the third was used to carry out a study which

compared the LSB and the conventional elutriate bioassay (CEB).

Elutriate Preparation

Conventional elutriate preparation (a 1:4 ratio of sediment to water) for use in biological impact assessment involves the production of a volume large enough to satisfy the requirements for the bioassay, as well as chemical analyses (Table 1). This can amount to volumes in excess of 2 litres, requiring as much as 1 kg of sediment in some cases (Munawar et al., 1983). Since sample size and number were severely limited, bulk elutriates could not be prepared. Therefore, sediment:water ratios were developed to produce the LSB elutriates (Table 2) with concentrations equivalent to those normally used with conventionally produced elutriates. Table 3 lists the sediment:water ratios that were developed for all samples. Whenever sample size permitted, a 1:4 or 1:6 treatment was prepared. Under normal conditions (Conventional Elutriate Bioassay), the elutriate would be added to incubation bottles containing either natural assemblages or cultured phytoplankton as test organisms (Munawar et al., 1983). However, in the LSB procedure, concentrated algal culture was added to the LSB elutriates in order that the required sediment:water ratios could be maintained.

For each treatment, a 200 ml LSB elutriate was prepared, which provided enough medium to produce four 50 ml replicates. Filtered Lake Diefenbaker water was used to prepare these

elutriates. To achieve this, sediment and water were mixed (Table 3) using a Kraft Apparatus Rugged Rotator ferris-wheel type tumbler at a rate of 5 rpm, for one hour (Daniels et al., 1986). Samples were removed and allowed to stand for six hours at 4°C, after which they were decanted and centrifuged at 10,000 rpm for 20 minutes. The supernatant was then filtered using 0.45 μm Millipore membrane filters, after which the resulting elutriates were then ready for use in bioassays.

Conventional elutriate was also prepared from the Riverhurst Ferry sediment (Munawar et al 1983), in order to generate elutriate data for comparison with LSB results. Elutriate from this sample was therefore available for chemical analysis.

Toronto Harbour Comparative Study

Sediment samples were collected from two sites (Station 6 and Station B) in Toronto Harbour in April 1986. Subsamples were then prepared by weight (wet) and used in the preparation of LSB elutriates as described above (Table 3). Sub-samples of these sediments were also used for chemical analysis.

Other comparative experiments were also conducted using a third Toronto Harbour sediment, collected in June 1986, in both LSB and CEB evaluations. As previously described, LSB and conventional elutriates were prepared for use in biological assessment.

Chemical Analysis

A limitation of the LSB method is that chemical analysis can be performed only on the solid phase material (suspended particulate matter or sediment), since the volume of elutriate produced exactly matches the bioassay requirement. Therefore, the levels of a variety of trace metals from the solid material were determined by the Water Quality National Laboratory of Environment Canada (Burlington, Ontario) using their standard atomic absorption and plasma emission spectroscopic methods (Water Quality National Laboratory 1985). The results are presented in Table 4.

Bioassays

In performing bioassays of the Western and Arctic elutriates, 1 ml of a mixed phytoplankton culture (laboratory-grown in Algal-Gro) containing approximately 6.8 x 10^4 cells, was added to each limited sample elutriate. (Algal-Gro is a sterile, nutrient-rich, freshwater, universal medium buffered at pH 7.5, and commercially produced by Carolina Biological Supply Co.). Culture preparation procedures (washing, isolating, etc.) used were those recommended by James (1978). Each 100 ml polycarbonate Erlenmeyer bottle contained 50 ml of the elutriate before phytoplankton inoculation. Species of Cyanophyta, Chlorophyta, Diatoms, and Euglenophyta were included in the mixture which was composed of the following species:

Anacystis nidulans Gardner
Ankistrodesmus sp.
Pandorina morum (Muell.) Bory
Carteria olivierie G.S. West
Synedra sp.
Trachelomonas sp.

While conducting the Toronto Harbour limited sample bioassays, however, 1 ml additions of a heavy monoculture of Chlorella vulgaris Beyerinck were applied to each 50 ml LSB elutriate incubation unit, instead of an algal mixture. During the LSB-CEB comparative study, each 50ml LSB elutriate was inoculated with 1.72 ml of C. vulgaris concentrate; whereas 50 ml of the same C. vulgaris concentrate were added to 1500 ml of 0.45μm filtered Lake Ontario water, well mixed, then used to provide 50 ml aliquots for each CEB replicate. LSB treatments consisted of sediment:water mixtures of 0:1, 1:36 and 1:4, while the CEB treatments were 0:1, 1:40 and 1:10. A 1:4 CEB treatment would have entailed using an incubation mixture made up entirely of the conventional elutriate, rather than a culture medium, to which had been added measured quantities of elutriate.

All incubation bottles were then given a treatment of 1 μCi each of ^{14}C as $NaHCO_3$ and incubated in a Conviron E7 growth chamber (with a CMP 2023 microprocessor) for 4 hours at 15°C and a constant light intensity of 238 $\mu E\ m^{-2}\ sec^{-1}$ at 400 - 700 nm. At the end of the incubation period, a 1 ml sub-sample was removed from each bottle before it was filtered onto 0.45 μm Millipore membrane filters at a pressure of 17.7 kPa. Each 1 ml aliquot was placed into a separate scintillation vial and preserved in monoethanolamine so that the total ^{14}C activity

actually available in each bottle could be determined. The cells collected on the filter in each case were washed with 10 ml 0.1N HCl while under vacuum pressure to remove unincorporated $NaH^{14}CO_3$ (Munawar et al., 1983). The filters were placed separately into scintillation vials. After all filtration was completed, each vial was then treated with 10 ml of scintillation fluor (PCS II, Amersham Corp., Arlington Heights, Illinois), agitated with a vortex mixer, and subjected to scintillation counting (Lind and Campbell, 1969).

The resulting bioassay data, which was adjusted to make corrections for dark carbon assimilation, provided a measure of the algal production in mg C m^{-3} h^{-1}. Statistical treatment of the data included the computation of means, standard errors and tests of significance using the Student's t-test.

RESULTS AND DISCUSSION

Not all data are presented in this paper, but samples from all three geographic areas are included. From Figure 2, it is evident that both Red Deer River SP samples had a consistent enhancement of carbon assimilation. The April sample produced statistically significant effects compared with the control at the three highest treatment levels only ($P<0.05$ to $P<0.001$). Significant enhancement in the June sample was even more progressive ($P<0.05$ to $P<0.001$) when compared with the control. The physiological response was similar in both samples even

though there were significant differences in Cu, Mn, Ni, Pb, and Zn concentrations (Table 4). It is likely that the nutrient levels of these sediment elutriates was high, which would account for the observed results. Alternatively, the degree of trace metal release during elutriation may have been low.

Conversely, the South Saskatchewan River SP sample exhibited a consistent inhibition of carbon uptake (Figure 2). Although the particulate sample size permitted only two treatment levels, a significant suppression of carbon assimilation was evident at the 1:16 level of treatment ($P<0.05$). Since the South Saskatchewan trace metal characteristics represent an approximate average of the two Red Deer samples (Table 4), the toxic pattern may be due to a lack of nutrients, increased availability of metals, and/or the presence of other contaminants not analysed in this investigation. Interpretations of the basic physiological effects indicated above are hampered by the absence of chemical data for the liquid phase. This is the principal limitation of the LSB technique, but it must be remembered that this approach is designed to salvage meaningful data from samples which would be otherwise useless for biological assessment by the elutriate technique.

Figure 3 shows the results of bottom sediment bioassays. The Fish Lake sample treatments caused significant inhibition ($P<0.01-0.001$) compared to the control. The available chemistry data for this sediment are comparable to those of the rivers and lakes of that area, but has less Cu, Mn, Pb and Zn than the

Toronto Harbour sites. Both of the Toronto Harbour sediment LSB-elutriates caused inhibition of productivity ($P<0.05$ - $P<0.01$) but only at the highest treatment levels (1:4). As apparent from Figure 3, a slight enhancement of the productivity was observed at lower doses in both samples, but these responses were not statistically significant. The significantly higher levels of Cr, Zn, Cu, and Pb observed in the Toronto samples (Table 4) may have resulted in the observed inhibition of carbon assimilation.

The Riverhurst Ferry (Figure 3) samples exhibited a consistent and significant enhancement of carbon assimilation at all treatment levels with uptake ranging from 17 to 27 percent ($P<0.001$). Metal concentrations in the Riverhurst Ferry sediment were generally similar to those of the SP samples except that Cr concentrations were elevated. As in the case of the Red Deer River SP bioassay, the enhancement observed may have involved elevated nutrient levels, decreased available metals, or both.

It is interesting to compare the results of the LSB experiment with the conventional elutriate bioassay (Figure 4) prepared from the same Riverhurst Ferry bottom sediment. A general enhancement of productivity was shown by the >20 μm size fraction. The productivity was significantly increased ($P<0.01$) at both the 5 percent and 10 percent treatment levels. On the other hand, the <20 μm size assemblage exhibited a increasingly toxic response by additions of the same elutriate ($P<0.05$ - $P<0.01$), thereby indicating differential responses of the two

types of size assemblages. The mixed culture of algae in the the LSB experiments responded to elutriate treatment in a manner which was similar to that of the >20 μm size fraction of the natural population, whilst the same elutriate was toxic to the sensitive <20 μm size fraction phytoplankton. These results are in agreement with Great Lakes observations reported by Munawar and Munawar (1987).

The Toronto Harbour sediment study comparing the relative performances of the LSB and the CEB techniques revealed (Figure 5) that generally comparable data were acquired by use of the two techniques in incubation systems which contained a C. vulgaris monoculture. In this instance, the general indications were that no significant biological effects were measured by either method. It should be acknowledged at this point that the LSB and CEB treatments were not of accurately comparable ratios, but the 1:36 and the 1:4 LSB treatments are roughly comparable to the 1:40 and 1:10 CEB treatments respectively being representative of "low" and "high" treatment levels. Therefore, the results show that the new LSB technique is both effective and comparable to the conventional elutriate procedure.

SUMMARY AND CONCLUSIONS

The results discussed above indicate that the Limited Sample Bioassay (LSB) technique was successfully applied. Preliminary assessments of the bioavailability of contaminants can thus be

reliably and inexpensively performed in considerably less time than with the conventional elutriate bioassay. A large proportion of the time required by the latter is consumed by the process of elutriation, settling, centrifugation, and filtration. These activities are sharply reduced in the LSB method; hence time and money are conserved.

This approach is particularly applicable to a situation where sample material, funding, and/or time are limited. It would be possible to screen large numbers of samples at a fraction of the cost in order to identify problem samples. Once such screening has been completed, more extensive assessment procedures, both biological and chemical, can then be applied to contaminated samples. Moreover, when there is a scarcity of sample material, this method can help ensure that at a minimum, meaningful indications are obtained concerning the impact of a given sediment on the status of phytoplankton in a specific ecosystem.

Even with its shortcomings (lack of chemical data from the liquid phase), the LSB technique provides the sediment researcher with a valuable tool. Since suspended particulate matter appears to be a principal transporter of aquatic contaminants (Golterman et al., 1983) and are capable of interacting with contaminants to make them available to aquatic organisms (Leppard and Burnison, 1983), there would appear to be a role for the LSB technique in future biological assessment programmes.

ACKNOWLEDGEMENTS

We wish to thank Mrs. I.F. Munawar for phytoplankton enumeration of the test samples, as well as Dr. R.L. Thomas (International Joint Commission) and Dr. G.G. Leppard (National Water Research Institute) for the review of the manuscript. We would also like to thank Lynda McCarthy and Janice Jones for their assistance in the preparation of the manuscript.

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FIGURE LEGEND

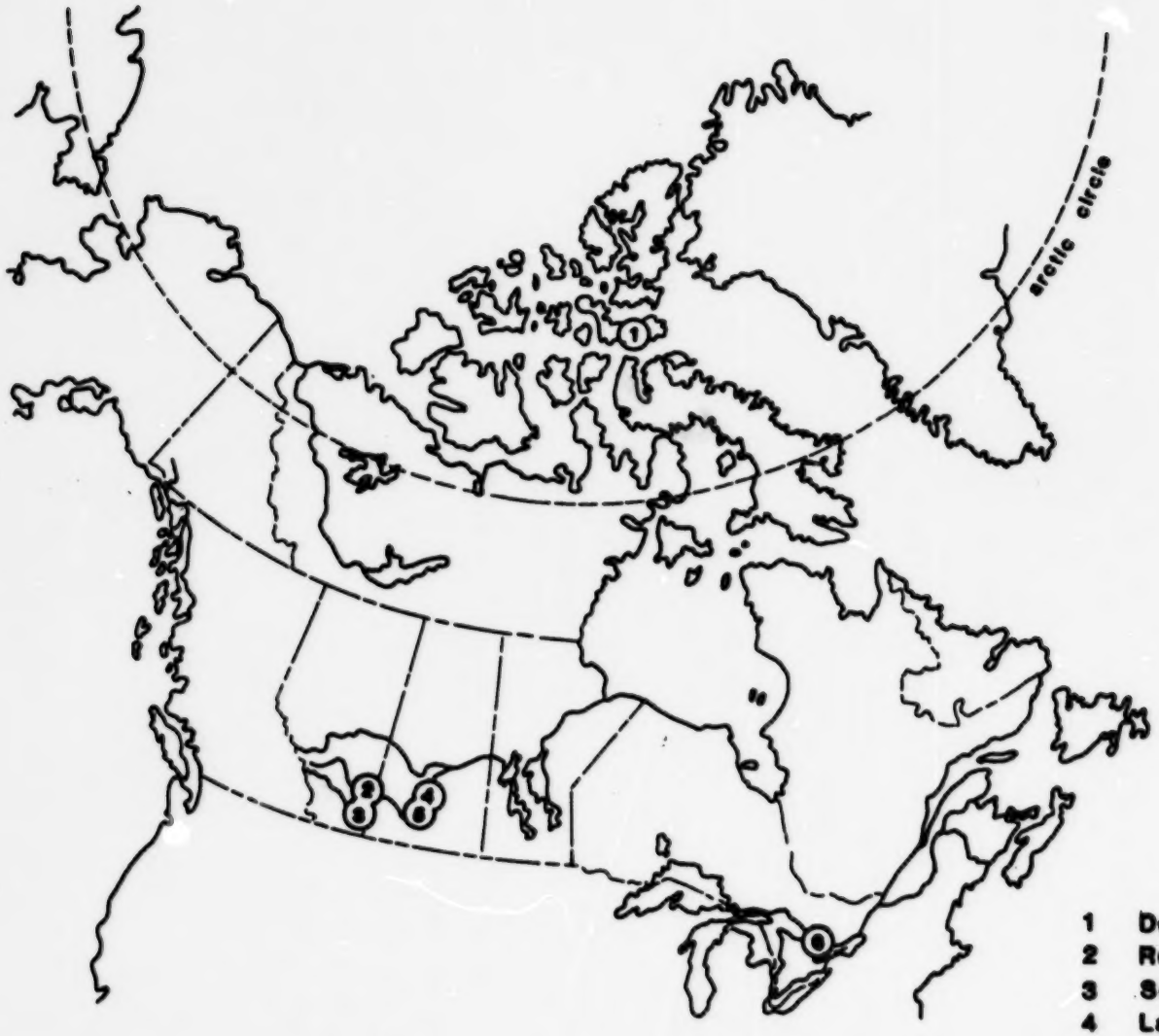
Figure 1. Map of Canada showing the location of sampling sites in western Canada (Alberta and Saskatchewan), the Northwest Territories, the Arctic (Devon Island), and central Canada (Toronto, Ontario).

Figure 2. Limited Sample Bioassays of suspended particulates from A: South Saskatchewan River, B & C: Red Deer River.

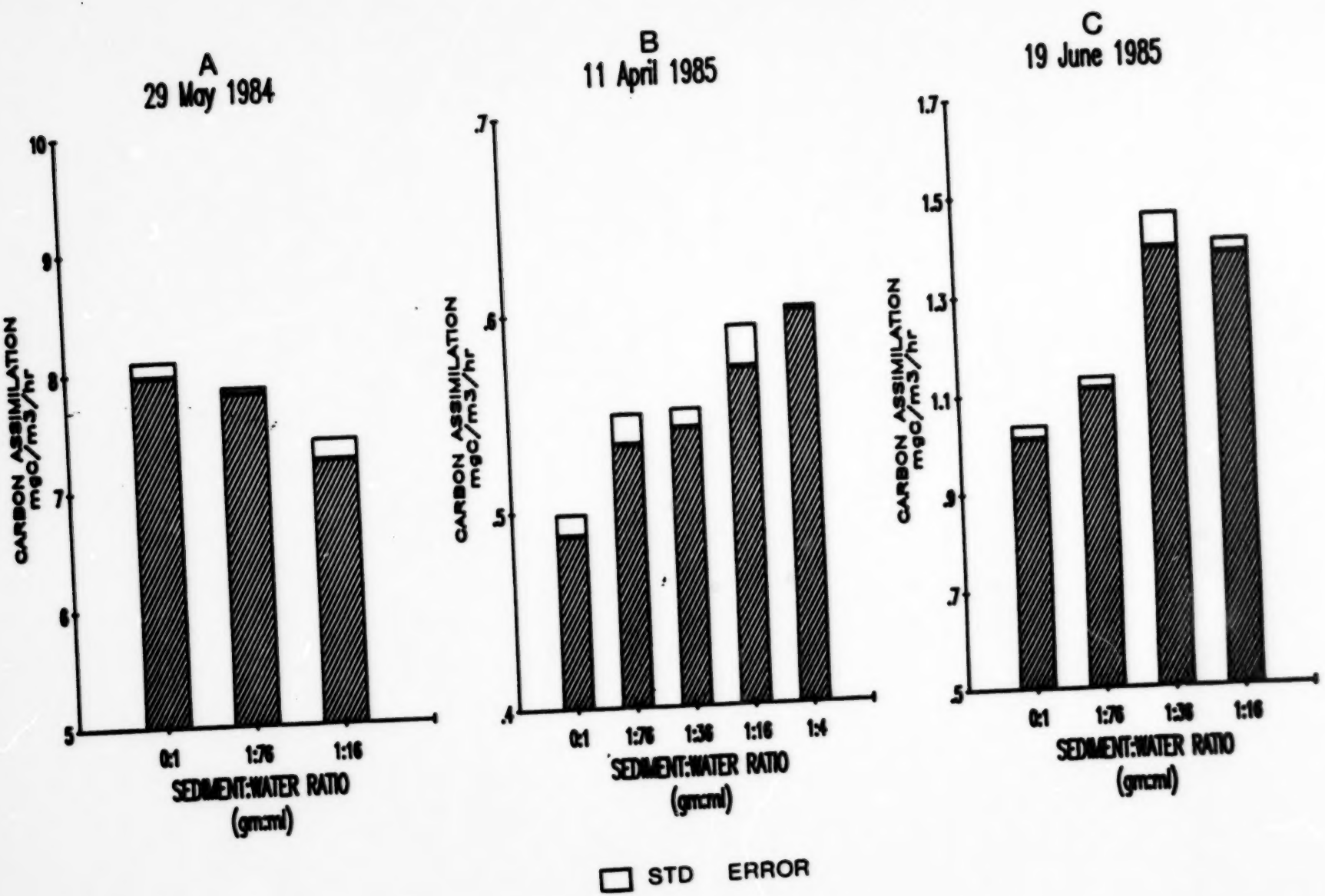
Figure 3. Limited Sample Bioassays of Fish Lake, Toronto Harbour (Inner Harbour), and Riverhurst Ferry sediments.

Figure 4. Conventional Elutriate Bioassays with Riverhurst Ferry sediment using two natural phytoplankton size assemblages.

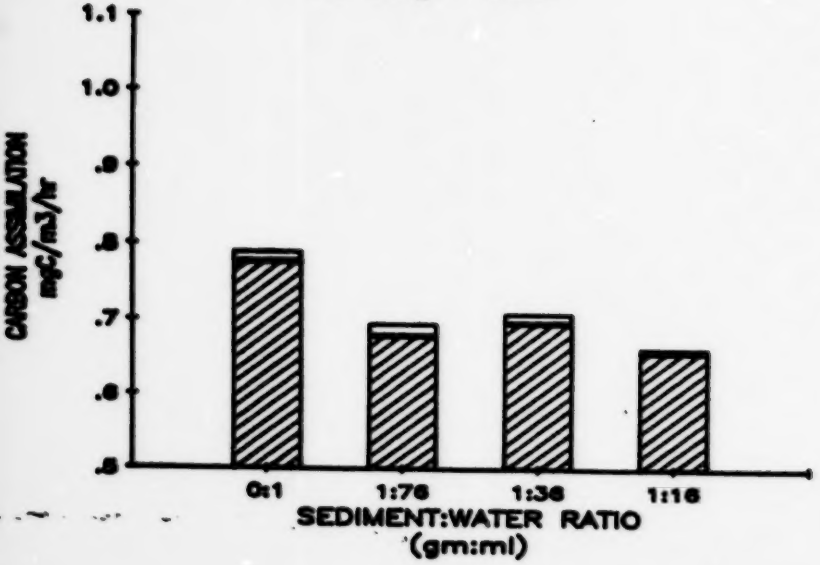
Figure 5. Limited Sample Bioassay and Conventional Elutriate Bioassay conducted with the same Toronto Harbour (mouth of Keating Channel) sediment collected in June 1986, using a laboratory-grown monoculture of Chlorella vulgaris, A: LSB data; B: CEB data.



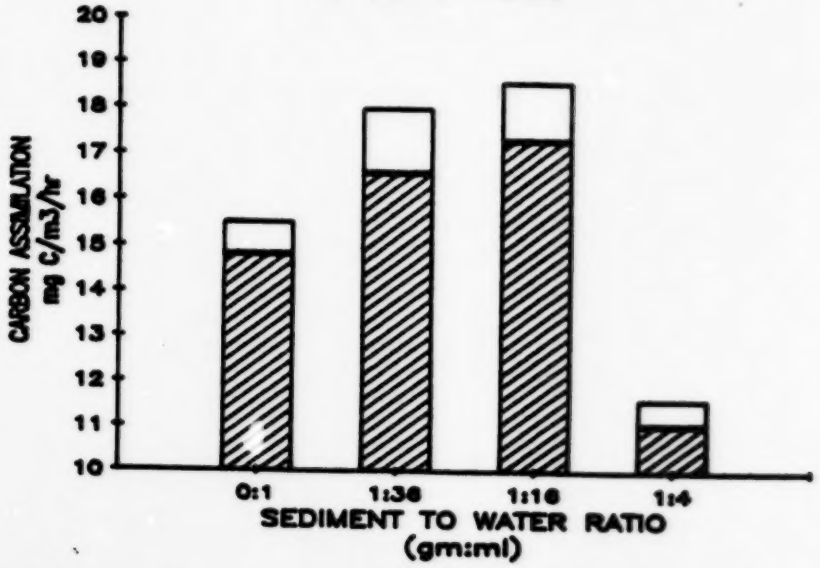
1 Devon Island
2 Red Deer River
3 South Saskatchewan River
4 Lake Diefenbacker at Elbow
5 Riverhurst Ferry
6 Toronto Harbour


A
29 May 1984
B
11 April 1985
C
19 June 1985
CARBON ASSIMILATION
mgC/m3/hr
10
9
8
7
6
5
.7
.6
.5
.4
1.7
1.5
1.3
1.1
.9
.7
.5
0:1
1:76
1:36
1:16
1:4
SEDIMENT:WATER RATIO
(gm:ml)
STD ERROR

FISH LAKE, DEVON ISLAND, N.W.T.
10 July 1985



STATION 6, TORONTO HARBOUR
12 June 1986



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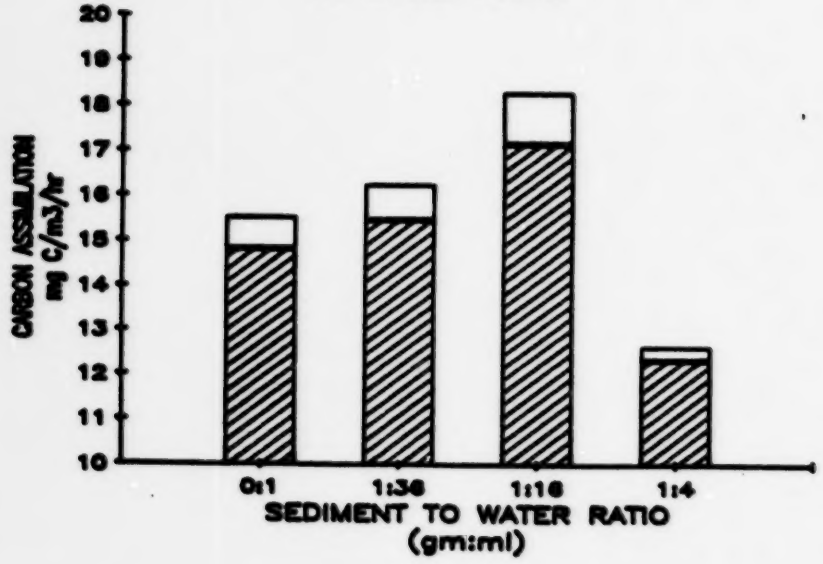
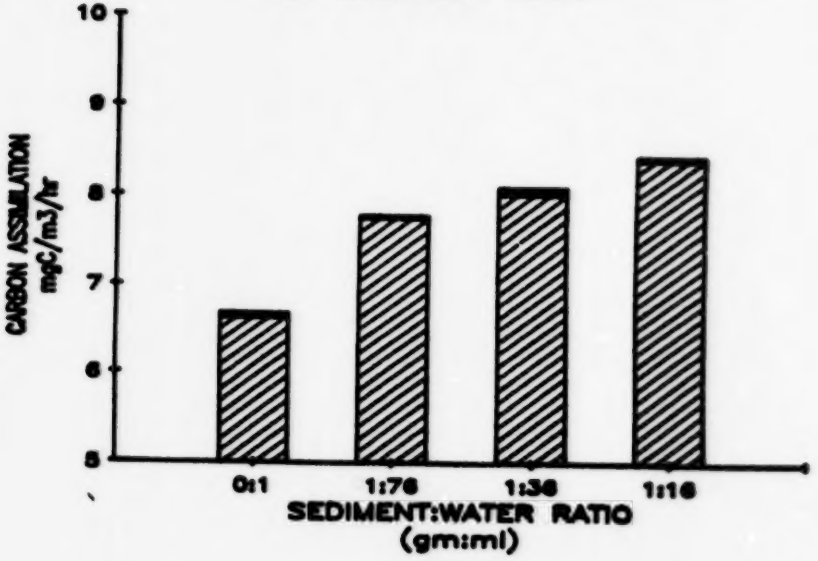
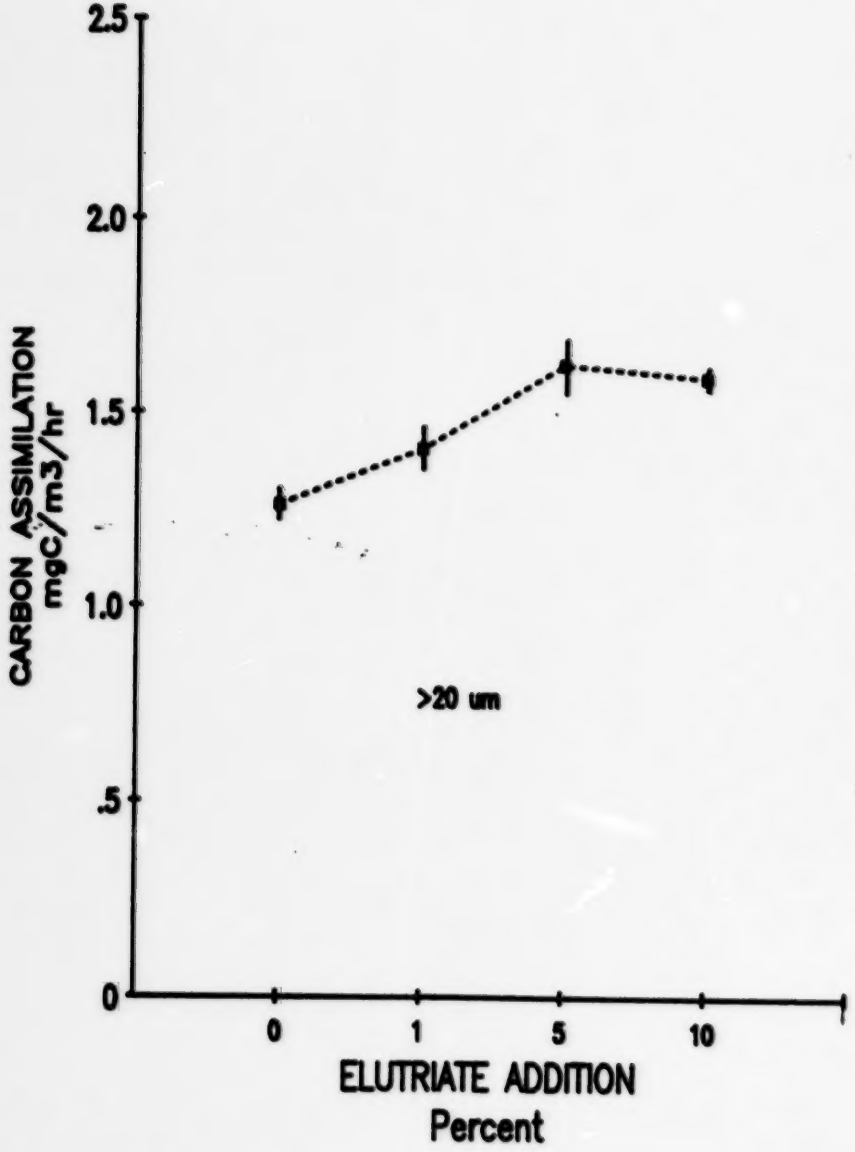
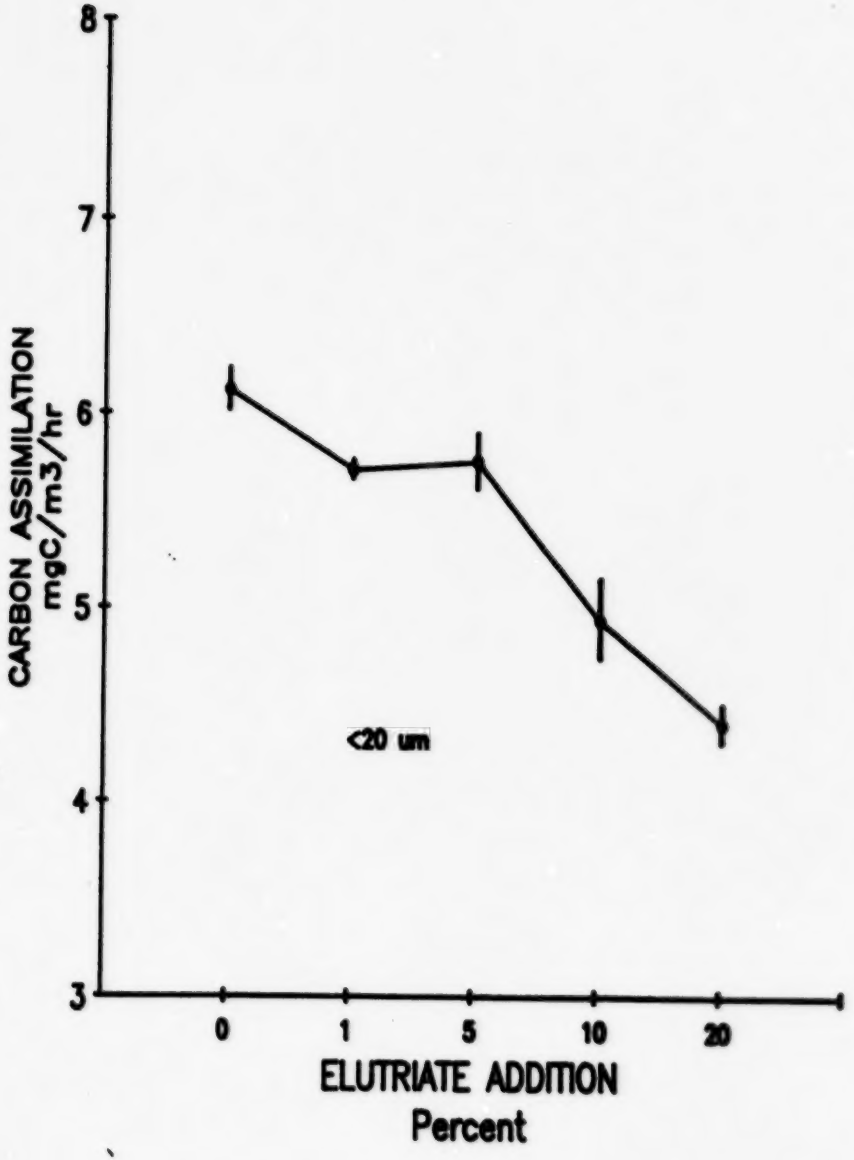


RIVERHURST FERRY, LAKE DIEFENBAKER
23 October 1985




2.5
2.0
1.5
1.0
.5
0
CARBON ASSIMILATION
mgC/m3/hr
>20 um
0
1
5
10
ELUTRIATE ADDITION
Percent


8
7
6
5
4
3
CARBON ASSIMILATION
mgC/m3/hr
<20 um
0
1
5
10
20
ELUTRIATE ADDITION
Percent

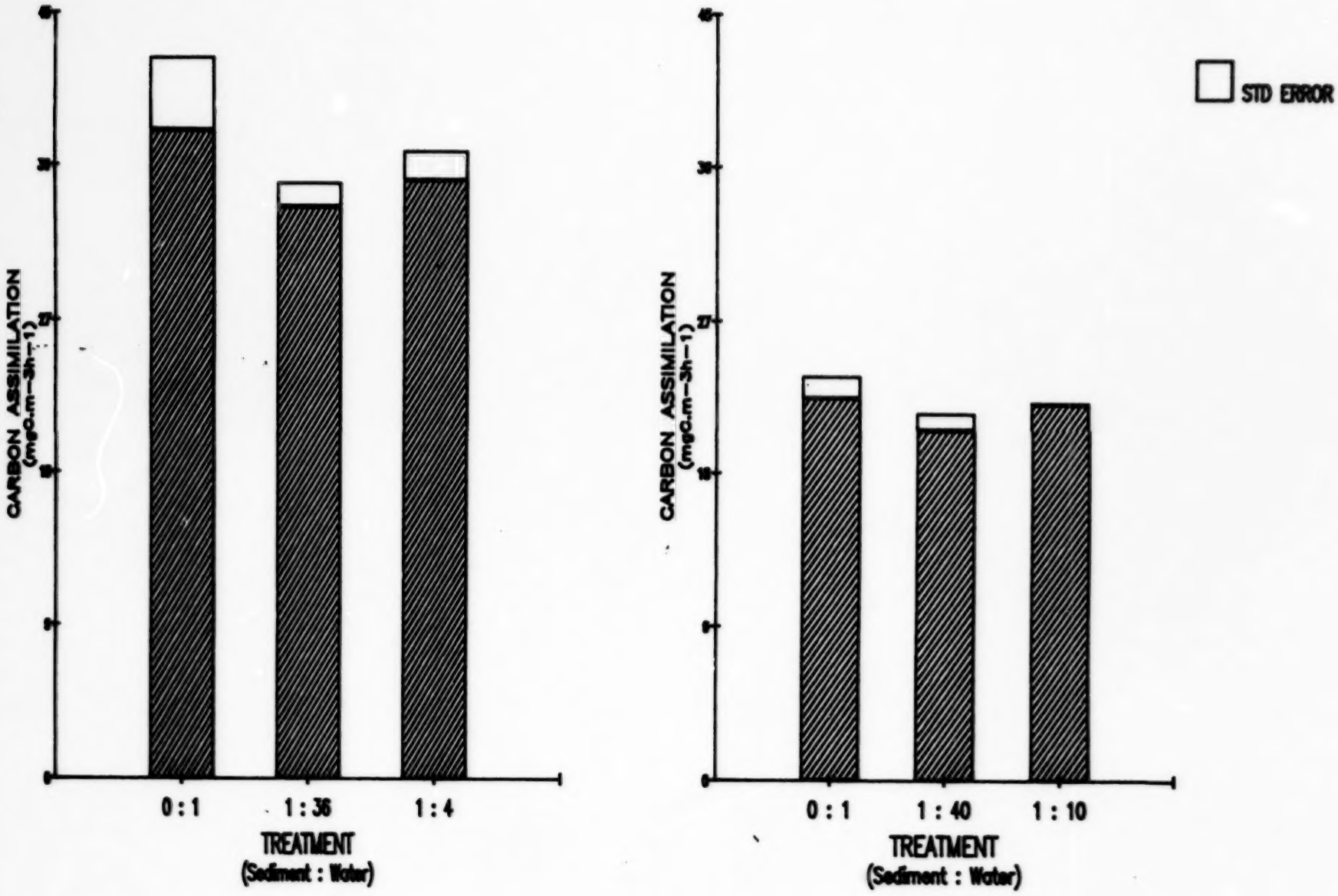

A
B
CARBON ASSIMILATION (mgC.m-3h-1)
STD ERROR
0 : 1
1 : 36
1 : 4
1 : 40
1 : 10
TREATMENT
(Sediment : Water)

TABLE 1: Sediment equivalents and sediment:water ratios resulting from the addition of various doses of standard elutriate (1:4 sediment:water) to natural phytoplankton.

Percent Addition	Sediment (in 200 ml)	Sediment:Water Ratio
Control	0.000	0 : 1
1	0.253	1 : 400
5	2.632	1 : 80
10	5.556	1 : 40
20	12.500	1 : 20
40	33.333	1 : 10

TABLE 2: LSB elutriate generation.

Sediment:Water gm : ml	Sediment:Water Ratio
0 : 200	0 : 1
2.632 : 200	1 : 76
5.556 : 200	1 : 36
12.5 : 200	1 : 16
33.333 : 200	1 : 6
50.0 : 200	1 : 4

TABLE 3: Sampling sites and treatments.

SAMPLE COLLECTION SITE (Abbreviation) Date of Collection	TREATMENT Sediment:water
Bottom Sediment Samples	
Lake Diefenbaker (off Elbow), Saskatchewan (Elbow) 85.09.18	1:76 1:36 1:16
Riverhurst Ferry, Saskatchewan (Riverhurst) 84.10.23	1:76 1:36 1:16
Loon Lake, Devon Island, Northwest Territories (Loon Lake) 85.07.09	1:76 1:36 1:16 1: 4
Fish Lake, Devon Island, Northwest Territories (Fish Lake) 85.07.10	1:76 1:36 1:16
Toronto Harbour, Ontario (Toronto-6) 86.06.12	1:36 1:16 1: 4
Toronto Harbour, Ontario (Toronto-8) 86.06.12	1:36 1:16 1: 4
Suspended Particulate Samples	
South Saskatchewan River, Saskatchewan (S. Sask. R.) 85.04.10	1:76 1:36 1:16
South Saskatchewan River, Saskatchewan (S. Sask. R.) 84.05.29	1:76 1:16
South Saskatchewan River, Saskatchewan (S. Sask. R.) 85.06.19	1:76 1:36 1: 6
Red Deer River, Alberta (Red Deer R.) 84.05.29	1:76 1:16
Red Deer River, Alberta (Red Deer R.) 85.04.11	1:76 1:36 1:16 1: 4
Red Deer River, Alberta (Red Deer R.) 85.06.19	1:76 1:36 1:16

TABLE 4: Trace metal content of suspended particulates and bottom sediments.

Sample Site	Type	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Se	V	Zn
S. Sask. R. 84.05.29	SP	58.9	9.8	<10	29.5	59.3	29.1	26.3	0.05	592	<50	20.9	<50	1.10	172	101
Red Deer R. 85.06.19	SP	47.4	9.1	<10	<20	--	37.9	29.2	0.06	719	50	<20	71	0.40	140	118
Red Deer R. 85.04.11	SP	52.1	--	10	20	--	17.0	21.5	0.07	400	—	40	50	--	100	59
Riverhurst 84.10.23	BS	48.5	9.0	<10	<20	86.6	31.6	31.7	0.07	698	<50	29.3	<50	0.70	169	112
Loon Lake 85.07.09	BS	--	—	--	--	--	--	--	—	—	--	--	--	--	—	--
Fish Lake 85.07.10	BS	--	--	--	--	--	--	--	--	—	--	--	—	--	--	--
Toronto-6 86.06.12	BS	46.7	3.79	<10	<20	73.9	81.8	28.1	--	701	--	24.9	201	0.35	--	466
Toronto-8 86.06.12	BS	45.1	3.85	<10	<20	91.0	110.3	28.5	--	664	--	<20	235	0.28	--	474

N.B. 1. All values are expressed as $mg.Kg^{-1}$
2. The values for Al and Fe should be $x10^{3}$
3. Values for Ni and Zn have been rounded-off
4. Values for Riverhurst Ferry are the means of two sets of data.
5. BS = Bottom Sediment SP = Suspended Particulate